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16. ABSTRACT
Seven chemicals commonly found at industrial waste sites were tested with the Tradescantia-micronucleus (Trad-MCN) assay to evaluate their clastogenic potential. They were: Aldrin, arsenic trioxide, 1,2-benz(a,h)anthracene, dieldrin, heptachlor, lead tetraacetate, and tetrachloroethylene. Results of repeated tests for clastogenicity yielded the minimum effective dose (MED) of 0.44 ppm for lead tetraacetate, 3.81 ppm for dieldrin, and 1.88 ppm for heptachlor. Arsenic trioxide and 1,2-benz-(a,h)anthracene yielded positive responses at the MED of 3.96 ppm and 2.28 ppm, respectively. Aldrin and tetrachloroethylene were considered to be immiscible with water, and the test yielded negative responses. Results of tetrachloroethylene fume treatment yielded a positive response at the MED of 30 ppm/min in 2-hr exposures. Five chemicals determined to be clastogens by this test were ranked according to their MED in the descending order of potency as follows: Lead tetraacetate, heptachlor, 1,2-benz(a,h)anthracene, dieldrin, and arsenic trioxide. Results of this study indicate that Trad-MCN bioassay could be effectively utilized for assessing the genetic hazard from the leachates and volatile compounds emanating from uncontrolled industrial waste sites.

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Clastogenicity evaluation of seven chemicals commonly found at hazardous industrial waste sites

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Summary

7 chemicals commonly found at the industrial waste sites were tested with the Tradescantia–Micronucleus (Trad–MCN) assay to evaluate their clastogenic potential. Chemicals selected from the US EPA Superfund Priority 1 list were: aldrin, arsenic trioxide, 1,2-benz[*a*,*h*]anthracene, dieldrin, heptachlor, lead tetraacetate and tetrachloroethylene. Results of repeated tests for clastogenicity yielded the minimum effective dose (MED) for clastogenicity of 0.44 ppm for lead tetraacetate, 1.88 ppm for heptaclor, 3.81 ppm for dieldrin and arsenic trioxide and 1,2-benz[*a*,*h*]anthracene yielded positive responses at the MED of 3.96 ppm and 12.50 ppm respectively. Aldrin and tetrachloroethylene were considered to be immiscible with water, and the tests yielded negative responses. Tetrachloroethylene in gaseous state was also used to treat the flower buds. Results of tetrachloroethylene vapor phase treatment yielded a positive response at the MED of 30 ppm/min after a 2-h exposure. 5 chemicals determined to be clastogens by this test were ranked according to their MED in the descending order of potency as follows: lead tetraacetate, heptachlor, dieldrin, arsenic trioxide and 1,2-benz[*a*,*h*]anthracene. Results of this study indicate that the Trad–MCN bioassay could be effectively utilized for assessing the potential clastogenicity of the chemicals commonly found at the industrial hazardous waste sites.

The chemicals abandoned at the industrial waste sites are the major environmental pollutants in the soil and water of the nearby area. The long-range effects of these hazardous wastes carried in the water table and accumulated in the aquafers are the worries for generations to come. Among more than a thousand known contaminants in the ground water near these waste sites, 7 chemicals were selected from the US EPA's Priority 1 chemical list (Waters et al., 1987) for laboratory tests using the Tradescantia–Micronucleus (Trad–MCN) bioassay. The main purpose of this investigation is to determine the "Minimum Effective Dose" (MED) of each of these chemicals under this test system. The extended aim is to validate the Trad–MCN bioassay for its capability to de-

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tect these individual chemicals at their relative low concentrations before testing the mixture forms (Yang and Rauchman, 1987) or conducting on site monitoring (Ma et al., 1984). Although Trad–MCN has been applied to ground water testing (Ma et al., 1987), surface water testing (Ma et al., 1985) and mutagenicity analysis of soil samples (Ho et al., 1983) as well as on site monitoring of air pollutants (Ma et al., 1982; Fang, 1981), a clastogenicity test of water and soil samples from the hazardous waste sites has so far not been conducted. The results of this study could determine the efficacy of the Trad–MCN bioassay for clastogenicity studies of the agents found at the hazardous waste sites.

Materials and methods

Plants of Tradescantia clone No. 4430 used in this investigation were raised in a greenhouse and were pre-conditioned in a growth chamber at a temperature range of 16–25°C and a 16/8 h (light/dark) photoperiod for 2 weeks or more before the cuttings were selected for conducting tests. The 7 chemicals used in this study were randomly selected from US EPA's Superfund Priority 1 list. Arsenic trioxide (CAS No. 7440-38-2 + 3), 1,2-benz[*a*,*h*]anthracene (CAS No. 53-70-3), dieldrin (CAS No. 60-57-1), lead tetraacetate (CAS No. 7439-92-1), and tetrachloroethylene (CAS No. 127-18-4) were obtained from Aldrich Chemical Company, 940 W. St. Paul, Milwaukee, WI 53233. Aldrin (CAS No. 309-00-2) was obtained from Crescent Chemical Inc., 1312 Motor Parkway, Hauauppauge, NY 11788 and heptachlor (CAS No. 76-44-8) from Chem Service Inc., P.O. Box 3108, West Chester, PA 19381. The purity level for these compounds was greater than 99%.

A series of preliminary experiments was conducted to determine the solubility in water, dimethyl sulfoxide (DMSO), ethanol (EtOH) and NaOH alkaline solution, as well as the optimal dose range for each of these chemicals under the pH range of 6–9. Since most of these chemicals were insoluble in water, aldrin, dieldrin, heptachlor, lead tetraacetate and tetrachloroethylene were first dissolved in DMSO and then diluted with water to the proper concentrations to treat the plant cuttings. Both aldrin and tetrachloroethylene were precipitated out after mixing with water. Arsenic trioxide was first dissolved in NaOH solution, and 1,2-benz[*a*,*h*]anthracene was dissolved in EtOH for treatments.

The plant cutting selection, treatment procedure, sample size, fixation, data collection, and analysis followed the conventional protocol as described in an earlier publication (Ma, 1983). As a rule, around 1500 tetrads per experimental group were scored to derive the MCN/100 tetrads frequencies. Analysis of variance and the Dunnett's *t* statistic were used to determine the significance of the difference among a series of treated against one control group. Since the chemicals used in the treatment solutions were in relatively low concentration, a modified procedure of 30 h continuous treatment period without recovery time was used (Ma et al., 1984, 1987). Most of the diluted water solutions for treatments were more-or-less in an emulsion state. Absorption of the very diluted emulsion through the vascular system of the plant cuttings was efficient enough to induce the chromosome damage judging by the cause/effect relationship (Ma et al., 1983a, 1984). Although Trad–MCN bioassay is primarily a genotoxicity test using the MCN frequency as the indicator of chromosome damage, the physiological injuries of the leaves and stem as well as the flower buds are also the reliable endpoints of toxicity and, at the same time, the signs of overdose. The overdose effects can also be expressed by the presence of dead cells in the tetrads (Ma et al., 1983a) and the decline of MCN frequencies. During the treatment period, the liquid mixtures were aerated by bubbling air through a fine tubing (2 mm in diameter). For inorganic chemicals, a $NaHSO_3$ solution was used as the positive control, and for the organic chemicals, benzo[*a*]pyrene was used as the positive control. Appropriate solvent controls were used to rule out the excessive clastogenicity of the solvents. A dynamic flow gas fumigation system (Ma et al., 1984) was used for gaseous treatment.

Results and discussion

Arsenic trioxide is used in manufacturing glass and enamel in addition to its extensive usage as a herbicide and an insecticide. The genotoxic effects of arsenic trioxide has been extensively evaluated

in a variety of in vitro and in vivo animal test systems. This compound yielded negative responses for gene mutation in prokaryotic systems (Rossman et al., 1980; Tiedmann and Einbrodt, 1982; Marzin and Phi, 1985; Hemmerly and Demerec, 1955), in Chinese hamster lung cells (Rossman et al., 1980), Syrian hamster embryo cells (Lee et al., 1985) and in the *Drosophila melanogaster* sex-linked recessive lethal assay (Dugatova, 1980). However, in most of the studies, this compound has produced positive results for chromosome aberrations in human lymphocytes (Nakamuro and Sayato, 1981; Oppenheim and Fishheim, 1965; Sweins, 1983; Wan et al., 1982; Larramendy et al., 1981; Nordensen et al., 1981; Zanzoni and Jung, 1980); in Syrian hamster embryo cells (Lee et al., 1985); in Drosophila for aneuploidy (Dugatora et al., 1980); in the mouse micronucleus test (Deknudt et al., 1986) and for chromosome aberrations in mouse bone-marrow cells (Sram, 1976). In the current study, arsenic trioxide was dissolved in NaOH solution at pH 7 in the lower concentrations and at pH close to 9 in the higher concentration. The results of treated samples at 4 concentrations, along with negative, positive and solvent control groups are given in Table 1.

Treatment with the dosage ranging from 0.2 ppm through 19.8 ppm gave a dose-related increase of MCN frequencies. Clastogenic responses given in this bioassay agree with the earlier findings on chromosome breaks in human lymphocytes, and SCE in cultured cells of human and Syrian hamsters (Larramendy et al., 1981; Nordenson et al., 1981; Nakamuro and Sayato, 1981).

The hazardous chemical, 1,2-benz[*a*,*h*]anthracene is a coal tar byproduct. It has been reported to covalently bound to DNA (Alfred and Dipaolo, 1968; Kuroki and Heidelberger, 1971; Duncan and Brooks, 1972; Bowden et al., 1974), and induced cell transformation (Sivak et al., 1980; Reznikoff et al., 1973; Hubermann et al., 1972; Pienta et al., 1977; Casto, 1973). The only clastogenicity evaluation of this compound was reported by Pal (1981) who claimed that the derivatives of this compound induced a high rate of SCE in cultured Chinese hamster ovary cells.

Ethanol was a better solvent for 1,2-benz[*a*,*h*]-anthracene than DMSO. The diluted EtOH solution of this chemical was utilized to treat the plant cuttings. Results of a series of treatments with increasing dosages, together with the negative and the solvent controls are given in Table 2.

Positive responses were demonstrated if the treated groups were compared with the negative control (water), but only T-2 and T-3 groups showed positive responses if they were compared with the solvent control (1.5% ethanol).

The biological effects of lead compounds in general have been studied through epidemiological approaches in occupationally-exposed populations (Al-Hakkak et al., 1986; Ding, 1987; Ichiba et al., 1987; Parkinson et al., 1987; Schmid and Bauchinger, 1972). Cytogenetic damage, physiological disturbance, blood pressure, and possibly

TABLE 1

CLASTOGENICITY OF ARSENIC TRIOXIDE DETECTED BY THE Trad-MCN BIOASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	NaOH	0.20 ppm	1.27	0.30	–	pH = 7
T-2	NaOH	1.98 ppm	6.70	4.64	+/–	pH = 7
T-3	NaOH	3.96 ppm	8.18	2.74	+	pH = 8
T-4	NaOH	19.80 ppm	36.99	10.41	+	pH = 9
C-W	water		1.20	0.26		negative control
C-S	NaOH	9.25 ppm [a]	2.03	0.71		solvent control
C-P	$NaHSO_3$ [b]	5.20 ppm	12.30	0.60	+	positive control

[a] Solvent only.

[b] Known clastogen.

TABLE 2

CLASTOGENICITY OF 1,2-BENZ[*a*,*h*]ANTHRACENE DETECTED BY THE Trad–MCN BIOASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	EtOH	2.5 ppm	5.69	1.40	–	
T-2	EtOH	12.5 ppm	6.24	1.51	+	
T-3	EtOH	25.0 ppm	8.92	1.13	+	
T-4	EtOH	50.0 ppm	5.87	0.85	–	overdose [a]
C-W	water		3.65	0.43		negative control
C-S	EtOH	1.5% [b]	4.30	0.85		solvent control
C-P	BaP	12.6 ppm	15.50	1.52	+	positive control

[a] Dead cells and physiological injuries of leaves and flower buds.
[b] Solvent only.

the cancer rate were affected by lead exposures in vivo. Lead acetate was reported to be a strong clastogen which breaks chromosomes in Chinese hamster ovary cells (Bauchinger and Schmid, 1972), in bone-marrow erythrocytes of rats (Kharchenko and Andreera, 1987; Tachi et al., 1985) and in cultured lymphocyte of humans. Chromatid aberrations were incuded by lead compounds of different valences at different rates (Gasiorek and Bauchinger, 1981). Kharchenko and Andreera (1987) claimed that spermatogenesis in rats was inhibited by lead acetate. Results of current tests on lead tetraacetate at 6 increasing dosages are given in Table 3. Positive responses were observed in lower dosages while the concentrations equal to or higher than 23.5 ppm were toxic and exhibited the overdose effects. The MED of this chemical was as low as 0.44 ppm for Trad–MCN test. Perhaps it was due to the high toxicity of this chemical that no positive linear dose-response could be obtained. Its range of clastogenicity was rather narrow. Although a great deal of attention has been paid to the hazardous nature of this group of simple organic lead molecules, more studies should be performed because of its high toxicity and clastogenicity.

Dieldrin, an insecticide, has shown no detectable mutagenic response in several prokaryotic and eukaryotic bioassays. In a few cytogenetic studies, i.e. mouse bone-marrow cells (Dean et al.,

TABLE 3

CLASTOGENICITY OF LEAD TETRAACETATE DETECTED BY THE Trad–MCN BIOASSAY

Expt. group	Solv.	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	DMSO	0.44 ppm	17.79	8.56	+	high variance
T-2	DMSO	2.35 ppm	18.93	2.90	+	
T-3	DMSO	11.75 ppm	13.07	5.50	+	
T-4	DMSO	23.50 ppm	3.34	0.43	–	overdose [a]
T-5	DMSO	44.00 ppm	2.81	0.31	–	overdose [a]
T-6	DMSO	88.00 ppm	2.20	0.67	–	overdose [a]
C-S	DMSO	0.25 ppm [b]	2.90	2.0		solvent control
C-W	Water		1.81	0.54		negative control
C-P	BaP	12.60 ppm	15.50	1.52	+	positive control

[a] Dead cells and physiological injuries of leaves and flower buds.
[b] Solvent only.

TABLE 4

CLASTOGENICITY OF DIELDRIN DETECTED BY THE Trad-MCN ASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	DMSO	0.38 ppm	1.35	0.4	−	
T-2	DMSO	3.81 ppm	9.80	1.8	+	
T-3	DMSO	19.05 ppm	3.40	0.6	−	overdose [a]
C-S	DMSO	0.25% [b]	2.90	2.0		solvent control
C-W	water		1.81	0.5		negative control
C-P	BaP	12.60 ppm	15.50	1.52	+	positive control

[a] Dead cells and physiological injuries of leaves and flower buds.
[b] Solvent only.

1975), the mouse dominant lethal assay (Dean et al., 1975, Epstein et al., 1972) and human lymphocytes in vivo (Dean et al., 1975), this compound yielded negative results. However, positive responses were obtained in studies on inhibition of intercellular communication of rodent cells in vitro (Kurata et al., 1982; Wade et al., 1980; Lin et al., 1986).

Positive response was obtained in the current study with the dieldrin treatment at around 3.81 ppm. The results are given in Table 4.

The chemical used in this test was in a 0.25% DMSO solution, and the solvent control group of this DMSO concentration gave a slightly elevated MCN frequency. A 5-fold increase of the MED gave an overdose response. Dead cells and dissynchrony of the meiotic stages of the pollen mother cells were observed in the overdosed group.

The insecticide heptachlor (heptachloro-tetrahydro-methanoindene) is known to be toxic and inhibitory to fetal development in rats (Yamaguchi et al., 1987). However, for gene mutation (Marshall et al., 1976) and DNA damage (Probst et al., 1981), heptachlor produced negative results except the waxy mutation in maize (Gentile et al., 1982). It also yielded negative response in the mouse dominant lethal assay (Epstein et al., 1972). However, this compound was positive for the inhibition of intercellular communication in rodent cells treated in vitro (Telang et al., 1982; Kurata et al., 1982). At the dosage ranging from 5 to 20 mg/kg to rat, reduced body weight and higher mortality rates were observed (Yamaguchi et al., 1987).

Current data which are given in Table 5 indicate high toxicity at around 18.8 ppm, and a short range of dose-related responses beginning at 1.88 ppm.

Both aldrin and tetrachloroethylene were immiscible with water although they were soluble in

TABLE 5

CLASTOGENICITY OF HEPTACHLOR DETECTED BY THE Trad-MCN BIOASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	DMSO	1.88	5.90	1.30	+	
T-2	DMSO	9.40	8.20	2.34	+	
T-3	DMSO	18.80	4.80	0.67	+/−	overdose [a]
C-S	DMSO	0.25% [b]	2.90	0.20		solvent control
C-W	water		1.88	0.35		negative control
C-P	BaP	12.60 ppm	12.60	1.52	+	positive control

[a] Dead cells.
[b] Solvent only.

TABLE 6

CLASTOGENICITY EVALUATION OF ALDRIN IN THE Trad–MCN ASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	DMSO	1.83 ppm	3.59	0.97	–	immiscible
T-2	DMSO	9.15 ppm	3.10	0.49	–	with water
T-3	DMSO	36.59 ppm	3.89	0.63	–	
C-S	DMSO	0.5% [a]	5.30	0.22		solvent control
C-W	water		4.26	0.52		negative control
C-P	BaP	12.60 ppm	15.50	1.52	+	positive control

[a] Solvent only.

DMSO but precipitated out after mixing with water. Thus, little absorption of these chemicals through the stem could take place. Negative responses were obtained in the dosages given in these tests as shown in Tables 6 and 7. A positive control group using benzo[*a*]pyrene was included in Tables 2–7, and serves as the reference for organic compounds in this study. Tetrachloroethylene was also tested under its gaseous state. Preliminary results are given in Table 7.

Clastogenicity studies of aldrin (an insecticide) conducted in human lymphocyte cultures (Georgian, 1975) and the MCN induction in vivo in mice and rats (Rani et al., 1980) showed positive results for chromosome aberrations but yielded no detectable response for micronuclei induction and for dominant lethal test in mice (Georgian, 1975).

Tetrachloroethylene (a volatile compound used in dry cleaning and for decreasing metals) is a well known neurotoxin. An epidemiological survey has been made in dry cleaning workers who were chronically exposed for a long duration (Bazylewicz-Walczak and Marszal-Wisniewska, 1986; Dudek and Nowachi, 1987). These indicated that this chemical affected the function of the psychomotor and central nervous system, and caused neurological disturbances. Ikeda et al. (1980) reported chromosome damage in cultured lymphocytes in humans.

Based upon the data obtained from Trad–MCN tests, 5 out of 7 chemicals were positive, and these clastogens can be ranked according to their MED in the following descending order of potency: lead tetraacetate (0.44 ppm), heptachlor (1.88 ppm), dieldrin (3.81 ppm), arsenic trioxide (3.96 ppm), 1,2-benz[*a*,*h*]anthracene (12.50 ppm). Based upon the study of Yang and Rauchman (1987), the average concentration of arsenic trioxide in the

TABLE 7

CLASTOGENICITY EVALUATION OF TETRACHLOROETHYLENE (IN LIQUID AND GASEOUS FORMS) IN THE Trad–MCN ASSAY

Expt. group	Solvent	Concentration	MCN/100 tetrads	S.E.	Signif. 0.05	Remarks
T-1	DMSO	150 ppm	1.22	0.26	–	immiscible
T-2	DMSO	300 ppm	4.99	0.62	–	with water
T-3	DMSO	600 ppm	3.23	0.92	–	
T-G		30 ppm/min/2 h	4.56	0.71	+	gaseous
C-G			1.90	0.25		gas control
C-S	DMSO	2% [a]	6.93	2.91	+/–	solvent control
C-P	BaP [b]	12.6 ppm	15.50	1.52	+	positive control

[a] Solvent only.

[b] Known clastogen.

ground water samples collected near the hazardous waste sites was about 7 times (30.60 ppm/3.96 ppm) higher than the MED obtained by the Trad–MCN bioassay, and the average lead concentration was about 80 times (37.0 ppm/0.44 ppm) higher than the MED. Results of the current study indicate that the Trad–MCN bioassay was capable to detect very low concentrations of clastogens in liquid forms if absorbed through the vascular system of the plant cuttings. It is a potential bioassay to assess the clastogenicity of chemicals commonly found at the industrial hazardous waste sites.

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